Effect of Temperature and Aggregation Rate on the Fractal Dimension of Renneted Casein Aggregates

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ABSTRACT

This study deals with the influence of chymosin/casein ratios and temperature on the fractal dimension of renneted casein aggregates in diluted milk. The angular dependence of static light scattering, from 50 to 110°, was used to determine fractal dimension from double logarithmic plots of intensity versus scattering vector. No significant effect was observed when varying chymosin/casein ratios from 3.3 to 79.2 μg of chymosin/g of casein at 30°C with a resultant fractal dimension value of 2.20. Temperature, on the other hand, influenced the aggregation mechanisms with fractal dimension values equaled to 2.32, 2.22, and 2.15 at 25, 30, and 35°C, respectively, for a chymosin/casein ratio at 13.2 μg of chymosin/g of casein. The importance of hydrophobic interactions in fractal aggregation of renneted casein particles is discussed.

(**Key words:** rennet, aggregation, casein, fractal)

Abbreviation key: D = fractal dimension, SLS = static light scattering.

INTRODUCTION

Aggregation of casein micelles in milk by acidification or/and renneting results in the formation of gels and curds largely used in the dairy industry, as in yogurt production or in cheese making. Casein micelles are colloidal particles described recently by the dual-binding model of Horne (1998) based on hydrophobic attraction and colloidal calcium phosphate bridging. The kappa-casein polyelectrolyte brush (De Kruif, 1996) permits stabilization of the micellar entity.

Understanding how hydrophobic and colloidal calcium phosphate bridging interactions can extend from the casein micelle scale to the gel scale implies analysis of each step of the aggregation and gelation phenomena.

The first step of casein micelle destabilization was determined using an adhesive hard sphere model. In this model, steric stabilization of casein micelles represented the most important stabilizing factor in milk (De Kruif, 1998; Tuinier and De Kruif, 2002). Then, aggregation and gelation of caseins occur according to fractal mechanisms demonstrated for acidification (Vétier et al., 1997; Chardot et al., 2002), renneting (Horne, 1987; Bremer et al., 1989; Lehner et al., 1999; Vétier et al., 2000), combination of renneting and acidification (Vétier et al., 1997; Mellema et al., 2000) and also for ethanol addition (Horne, 1987), and high temperatures (Walstra, 1990).

In previous works, we studied the influence of various parameters, i.e., temperature, rate of acidification and dilution, on the fractal aggregation of casein particles induced by acidification (Vétier et al., 1997; Chardot et al., 2002). As temperature was shown to be preponderant in determining fractal structures of acid casein aggregates, we chose, in this paper, to evaluate the influence of temperature and casein/chymosin ratios on the fractal dimension of renneted casein aggregates in unheated milk.

MATERIALS AND METHODS

Renneting of Milk

Skim milk was prepared from low-heat reconstituted nonfat dry milk (12% wt/vol) (Ingredia, Arras, France). Sodium azide was added to prevent bacterial growth (0.02%, wt/vol). The reconstituted nonfat dry milk was diluted 50-fold (vol/vol) in a permeate obtained from milk ultrafiltration using a membrane with a cutoff of 100,000 Da (membrane DC 10 LA; Amicon, Epernon, France). Temperature was controlled at 25, 30, and 35°C ($\pm 0.2^\circ\text{C}$).

Rennet aggregation of milk was provided by the addition of a chymosin solution. The concentration of chymosin solution was adjusted to get enzyme: substrate ratios equaled to 3.3, 13.2, 19.8, and 79.2 μg of chymosin/g of casein. Each experiment was replicated five times.

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Absorbance Measurements

Change in absorbance of renneted skim milk was monitored at 400 nm using a UV-Visible spectrophotometer (Ultrospec III; Pharmacia, Cambridge, England). Glass optical cells with path length of 1 mm were used, because of the concentration of skim milk solution.

Determination of Casein Particle Size

Variation of casein particle size during renneting was measured by dynamic light scattering with a 5-mW He-Ne laser ($\lambda = 633$ nm; Malvern Zetasizer III; Malvern Instruments, Worcestershire, England). Preliminary studies showed that no multiple scattering occurred in milk that had been diluted 50-fold (Vétier et al., 1997). Temperature was controlled at 25, 30, and 35°C ($\pm 0.2^\circ\text{C}$) by a Joule-Peltier device.

Determination of Aggregation Rates by Renneting

Aggregation time, which is a measurement of the onset of aggregation was defined by the time when casein particles size become larger than native casein micelles size. Aggregation rate of casein particles was obtained from the linear portion of the curves for absorbance versus time from the onset of aggregation (Vétier et al., 1997).

Determination of the Fractal Dimension for Casein Aggregates

The procedure was adapted from the static light scattering (SLS) procedure used by Raper and Amal (1993) that depends on the power law relationship between the intensity, I , scattered by a fractal structure and the magnitude of the momentum vector, Q :

$$I(Q) \propto Q^{-D},$$

where Q is a function of the scattering angle, $Q = (4\pi n/\lambda) \sin(\theta/2)$, λ is the wavelength of laser light and n , the refractive index of the medium.

The mean scattered intensity of light (expressed in photocounts) was measured at scattering angles from 50 to 110° and for 5 s for each angle. The fractal dimension (D) was then determined from double logarithmic plots intensity versus wave-vector, Q . These experiments were carried out at 25, 30, and 35°C just before the sedimentation of the casein aggregates when sizes of particles were sufficiently larger than the primary particles. Absorbance curves allowed the determination of the period of D measurements (i.e., just before the decline in absorbance; Horne, 1987).

Determination of the fractal dimension for casein aggregates according this procedure was validated by checking that time-dependent changes in scattered intensity were sufficiently slow so as to not interfere with the measurements of fractal dimension.

All experiments were conducted at least five times to ensure repeatability.

RESULTS

Influence of the Aggregation Rate on the Fractal Dimension of Renneted Casein Aggregates

The general shape of absorbance and size curves versus renneting time was not affected by variation of chymosin/casein ratios ranging from 3.3 to 79.2 μg of chymosin/g of casein; however, the rate and the onset of aggregation of casein particles was greatly influenced (Table 1).

Casein aggregates, obtained in these conditions, showed by SLS a linear relationship between the intensity, I , scattered and the momentum vector, Q . This demonstrates the fractal nature of renneted casein aggregates. The fractal dimensions determined at 30°C as a function of the chymosin/casein ratios, i.e., 3.3, 13.2, 19.8, and 79.2 μg of chymosin/g of casein, are equal to 2.21, 2.22, 2.23, and 2.21, respectively (Table 1). Considering the standard deviations, the fractal dimensions of casein aggregates obtained should be considered constant at about 2.20 under our experimental conditions.

Table 1. Aggregation time, aggregation rate, and fractal dimension of renneted casein aggregates obtained at 30°C by various Chymosin:casein ratios ($n = 5$).

Chymosin:casein ratio (μg chymosin/g casein)	Aggregation time		Aggregation rate		Fractal dimension	
	(min)	SE	(10^{-5} uDO $\cdot\text{s}^{-1}$)	SE	X	SE
3.3	1380	90	0.14	0.02	2.21	0.01
13.2	360	15	0.53	0.06	2.22	0.02
19.8	270	10	0.56	0.06	2.23	0.01
79.2	100	5	1.2	0.1	2.21	0.03

Table 2. Fractal dimension of renneted casein aggregates formed at 25, 30, and 35°C for a chymosin:casein ratio equaled to 13.2 μg of chymosin/g casein ($n = 5$).

Temperature (°C)	Fractal dimension	
	X	SE
25	2.32	0.01
30	2.22	0.02
35	2.15	0.02

Influence of Temperature on the Fractal Dimension of Renneted Casein Aggregates

A chymosin/casein ratio equaled to 13.2 μg of chymosin/g of casein was chosen to study the influence of temperature from 25 to 35°C on the aggregation of renneted casein particles. Linear relationships between I and Q permitted us to determine fractal dimension values of 2.32, 2.22, and 2.15 at 25, 30, and 35°C, respectively (Table 2).

DISCUSSION

In this work, we observed that temperature influenced the largely fractal structure of renneted casein aggregates, whereas no significant effect on D values was noticed for various chymosin/casein ratios. This was similarly demonstrated for acid aggregation of casein particles where temperature induced variations of fractal dimensions for acid casein aggregates, while aggregation rate was demonstrated to not have influence (Vétier et al., 1997).

Horne (1987) and Bremer et al. (1989) found values at around 2.4 for renneted casein aggregates in diluted milks. Our D values for renneted aggregates are 2.32,

2.22, 2.15, at 25, 30, 35°C, while acidified aggregates were defined by D values of 2.44, 2.36, 2.26, and 2.11 at 10, 20, 30, and 40°C (Vétier et al., 1997). We can notice that lower fractal dimension values, between 1.9 and 2.0, were found by Lehner et al. (1999) for enzymatically induced casein micelles aggregates in undiluted milk and by Chardot et al. (2002) for acidified casein aggregates in diluted milk. The difference in fractal dimension may come from distinct angular range of detection, from 50 to 110° in this study and from 0.01 to 50° in the studies of Lehner et al. (1999) and Chardot et al. (2002). We hypothesize that smaller aggregates are accessible with the 50 to 110° angular range, and that higher D values should indicate a more compact structure.

In Figure 1, D values for casein aggregates made by acidification and renneting show a similar dependence with temperature. Hydrophobic interactions should be then considered as influencing aggregation of casein particles destabilized either by acidification or renneting. By favoring hydrophobic interactions at higher temperatures, one can expect more stiffness of the junctions and less restructuring, leading to ramified aggregates with reduced fractal dimensions. Conversely, by inducing weak bonds, lower temperatures would permit rearrangements to a more completely filled structure for casein aggregates characterized by higher fractal dimensions (Vétier et al., 1997).

Temperature is also important in determining casein particles voluminosity whose influence on fractal dimension was suggested by Vétier et al. (2000) in the case of combined effects of acidification and renneting in casein aggregation. According to Mellema (2000), the decreased voluminosity with rising temperature gives

Figure 1. Fractal dimensions of casein aggregates as a function of temperature obtained from milk renneting with a chymosin/casein ratio equaled to 13.2 μg of chymosin/g casein (◆); milk acidification by 2% GDL (■) ($n = 5$). Milk is diluted 50-fold in permeate.

less deformable micelles with a decreased tendency to fuse together.

CONCLUSION

The aggregation of casein micelles induced by renneting gave rise to fractal aggregates. The influence of temperature was demonstrated: Increasing temperature and then hydrophobic interactions led to aggregates with reduced fractal dimensions. The *D* values and the *D* dependence with temperature were similar for acid and renneted aggregates. In the concentration range studied, the level of chymosin did not influence the fractal dimension of casein aggregates as it was the case for the level of acidifying agent (GDL) for acid casein aggregates in a previous study (Vétier et al., 1997).

All of these experiments were conducted on highly diluted milk samples (50×) and did not permit us to differentiate casein aggregates formed by two distinct mechanisms, acidification and renneting. In a real milk condition, where the protein concentrations are much greater, the question may be how relevant the structure is to differentiate an acid gel from a renneted gel. From our results, temperature rather than aggregation process may be considered as the major factor acting on casein aggregates structure.

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